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Lack of Action of Influenza Virus upon Mucin of Human or Swine Origin.*

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Francis[1] reported that normal serum in high dilution could inhibit hemagglutination by heated influenza virus. Burnet, McCrea, and Anderson[2,3] showed that (a) receptor destroying enzyme (RDE) of *Vibrio comma* and unheated influenza virus could destroy this inhibitor in normal serum, (b) a dilute solution of mucin derived from a pseudomucinous ovarian cyst and purified blood group substance A or O of human origin could inhibit hemagglutination by heated LEE influenza virus, and (c) RDE and unheated LEE virus could destroy this activity of mucin. The present report concerns the activity of influenza virus in the presence of purified preparations of human salivary mucin and gastric mucin of the hog.

The virus preparations were in the form of freshly harvested allantoic fluids of high titer derived from the infected chick embryo. The effect of various concentrations of human salivary mucin on 4 or 5 agglutinating doses of virus is recorded in Table I. The results show that as much as 2.5 mg/ml of mucin did not inhibit hemagglutination by heated or unheated influenza virus. No inhibition of hemagglutination was noted when gastric mucin of the hog was tested under the same conditions using swine influenza virus as well as the PR8 and LEE strains.

Since the salivary and gastric mucins did not inhibit hamagglutination, the technic used by Burnet, McCrea, and Anderson[3] could not be employed to determine whether or not influenza virus had any effect on mucin. However, an alternate technic utilizing the photoelectric colorimeter as a measure of turbidity was employed. In this type of experiment a small quantity of mucin in buffered saline was added to freshly harvested influenza virus. The results show that there was no decrease in the turbidity produced by mucin as a result of viral action (Table II). In addition the effect of the PR8 virus on both human salivary mucin and hog gastric mucin was studied to determine whether the virus could modify the conductivity or viscosity of mucin. In the conductivity test, there was no change in conductivity when 20 ml of PR8 virus with a final agglutinating titer of 1 to 40 acted on a solution of hog mucin or human salivary mucin containing 0.2 mg/ml of mucin in the presence of .0067M phosphate buffer at pH 7.4 at 25°C for 105 minutes and 204 minutes respectively. Using the Ostwald viscometer no alteration in viscosity of mucin was detectable in the presence of PR8 virus with a final agglutinating titer of 1 to 25 in .067M phosphate buffer at pH 7.4 when the virus acted on a 1% concentration of hog mucin and 0.25% concentration of human salivary mucin for 540 and 1017 minutes at 25°C respectively. These results are not surprising in view of the fact that Burnet, McCrea, and Anderson[2] have shown that the serological specificity of mucin is not destroyed by virus or by RDE. However, the clot or pellicle formed when a drop of the human salivary mucin or the gastric mucin was deposited in acid alcohol was dissolved by a drop of RDE thus indicating that these mucins were suitable substrates for RDE.

Two types of experiment were carried out to determine whether or not salivary or gastric mucin could inhibit the multiplication of in-

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1 Francis, T., Jr., *J. Exp. Med.*, 1947, **85**, 1.

2 Burnet, F. M., McCrea, J. F., and Anderson, S. G., *Nature*, 1947, **160**, 404.

3 Burnet, F. M., McCrea, J. F., and Anderson, S. G., *Austral. J. Exp. Biol.*, in press.

TABLE I.
Effect of Salivary Mucin on Hemagglutination by Viruses.

Virus	AD* of virus	Mucin mg ml	Hemagglutination Dilution of mucin 4	8	16	32	64	128	256	512	1024	Mucin control
PR8	4	2.5	+	+	+	+	+	+	+	+	+	—
PR8 heated 56°C/10	4	2.5	+	+	+	+	+	+	+	+	+	—
SW	5	10	+	+	+	+	+	+	+	+	+	—
SW ,, ,,	5	10	+	+	+	+	+	+	+	+	+	—
LEE	5	10	+	+	+	+	+	+	+	+	+	—
LEE ,, ,,	5	10	+	+	+	+	+	+	+	+	+	—

* AD—Agglutinating doses.

TABLE II.
Turbidimetric Readings of Virus-Mucin Mixtures.

Materials employed in test: Mucin mg/ml	LEE virus ml	NCAF ml	Saline ml	Photoelectric colorimeter reading Time in hrs at 37°C: 0	1.5	3.25	5	20
2.5	—	—	10	103	110	110	125	101
2.5	5	—	5	103	96	96	107	132
2.5	5	—	5	106	100	96	109	131
2.5	—	5	5	106	95	97	109	108
2.5	—	5	5	95	90	89	97	101
—	5	—	5	42	44	43	43	57

TABLE III.
Effect of Gastric Mucin on Infection of the Chick Embryo with LEE Virus

50% E.I.D.* of virus	Mucin mg/ml	No. eggs	RBC agglutination following inoculation: 48 hrs after	72 hrs after
1	—	6	3/6	4/6
	12.5	6	1/6	2/6
10	—	6	4/6	4/6
	12.5	5	5/5	5/5
100	—	6	6/6	6/6
	12.5	6	2/6	3/6
1000	—	6	6/6	6/6
	12.5	5	5/5	5/5

* E.I.D.—Egg infective dose.

fluenza virus in the developing chick embryo. In Table III are recorded the results obtained when the dilution of virus used in the inoculum is prepared in a solution of mucin containing 12.5 mg/ml of mucin and the mixture held at room temperature for one hour prior to inoculation of 0.05 ml into the allantoic cavity. In the other type of experiment one ml of allantoic fluid was withdrawn from each egg to be inoculated and one ml of the mucin solution injected into the egg one hour prior to inoculation with 0.05 ml of the virus dilution (Table IV). The results recorded in Table III and Table IV show that there is no inhibition of influenza virus multiplication when as much as 25 mg of mucin is introduced into the allantoic cavity prior to inoculation with ten 50% infective doses.

Comment. The evidence presented by Burnet and his colleagues[2,3,4] strongly sug-

[4] Burnet, F. M., *Lancet*, 1948, **1**, 7.

TABLE IV.
Effect of Salivary Mucin on Infection with LEE and PR8 Viruses.

50% E.I.D.* of virus	Mucin mg	Time mucin given in relation to virus inoculation	No. eggs	RBC agglutination 48 hrs after inoculation
100 LEE	5	1 hr before	5	5/5
100 "	—	—	6	6/6
10 PR8	25	1 " "	5	4/5
10 "	—	—	6	6/6

* E.I.D.—Egg infective dose.

gested that influenza virus has a direct enzymic action on mucin in the form of purified blood group substances. Burnet[5] thinks that the chief significance of this finding is that it provides a partial basis for the specific localization of infection by viruses of the mumps-influenza group to glands and mucous surfaces. Our failure to secure any evidence of viral action on human salivary mucin and hog gastric mucin indicates that the chemical composition and/or the molecular configurations of mucins derived from different sources are not identical. Supporting evidence for this viewpoint is contained in a recent report which shows that there are wide variations in the physical characteristics including the isoelectric point of mucin obtained from various levels of the gastro-intestinal tract of the hog.[6] The alteration of the mucinous blood group substances does not result in any alteration of the serological specificity of mucin[2] which again suggests that the change might involve only a slight molecular rearrangement.

Summary. Human salivary mucin and hog gastric mucin do not inhibit the hemagglutination induced by the PR8, LEE, and Swine strains of influenza virus and these mucins do not inhibit the multiplication of PR8 or LEE in the developing chick embryo. There was no turbidimetric or viscosimetric evidence of viral action on these mucins. Influenza virus was also unable to modify the conductivity of the human salivary mucin or the hog gastric mucin.

[5] Burnet, F. M., *Austral. J. Science*, 1947, **10**, 21.

[6] Domini, G., *Arch. fisiol.*, 1941, **41**, 36.

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Effects of Ether and Nembutal Anesthesia upon Blood Concentration of the Rat.

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The effects of ether and nembutal anesthesia on the blood concentration have been reported by several investigators. Anesthesia by nembutal results in hemodilution in cats[1] and in dogs.[2,3] Anesthesia by ether has been found to produce hemoconcentration in dogs[4-7] and

[1] Hamlin, H., Essex, H. E., and Mann, F. C., *Am. J. Physiol.*, 1939, **125**, 713.

[2] Hausner, E., Essex, H. E., and Mann, F. C., *Am. J. Physiol.*, 1938, **121**, 387.

[3] Hahn, P. F., Bale, W. F., and Bonner, J. F., Jr., *Am. J. Physiol.*, 1943, **138**, 415.

[4] Barbour, H. G., and Bourns, W., *Am. J. Physiol.*, 1924, **67**, 399.

[5] McAllister, F. F., *Am. J. Physiol.*, 1937, **119**, 363; 1938, **124**, 391.

[6] Bollman, J. L., Svirbely, J. L., and Mann, F. C., *Surgery*, 1938, **4**, 881.

[7] Searls, P. W., *J. A. M. A.*, 1939, **113**, 906.